Relationship Between EGFR Expression, *EGFR* Mutation Status, and the Efficacy of Chemotherapy Plus Cetuximab in FLEX Study Patients with Advanced Non–Small-Cell Lung Cancer

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Introduction: The phase III FLEX study (NCT00148798) in advanced non–small-cell lung cancer indicated that the survival benefit associated with the addition of cetuximab to cisplatin and vinorelbine was limited to patients whose tumors expressed high levels of epidermal growth factor receptor (EGFR) (immunohistochemistry score of ≥200; scale 0–300). We assessed whether the treatment effect was also modulated in FLEX study patients by tumor *EGFR* mutation status.

Methods: A tumor mutation screen of *EGFR* exons 18 to 21 included 971 of 1125 (86%) FLEX study patients. Treatment outcome in low and high EGFR expression groups was analyzed across efficacy endpoints according to tumor *EGFR* mutation status.

Results: Mutations in *EGFR* exons 18 to 21 were detected in 133 of 971 tumors (14%), 970 of which were also evaluable for EGFR expression level. The most common mutations were exon 19 deletions and L858R (124 of 133 patients; 93%). In the high EGFR expression group (immunohistochemistry score of ≥200), a survival benefit for the addition of cetuximab to chemotherapy was demonstrated in patients with *EGFR* wild-type (including T790M mutant) tumors. Although patient numbers were small, those in the high EGFR expression group whose tumors carried *EGFR* mutations may also have derived a survival benefit from the addition of cetuximab to chemotherapy. Response data suggested a cetuximab benefit in the high EGFR expression group regardless of *EGFR* mutation status.

Conclusions: The survival benefit associated with the addition of cetuximab to first-line chemotherapy for advanced non–small-cell lung cancer expressing high levels of EGFR is not limited by *EGFR* mutation status.

Key Words: Cetuximab, EGFR expression, EGFR mutation, Chemotherapy, Advanced NSCLC, FLEX.

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The phase III FLEX study showed that the addition of the epidermal growth factor receptor (EGFR) antibody cetuximab to cisplatin and vinorelbine chemotherapy significantly improved overall survival compared with chemotherapy alone in the first-line treatment of patients with EGFR-expressing advanced non–small-cell lung cancer (NSCLC).[1] Prospectively collected EGFR immunohistochemistry (IHC) data from FLEX study patients subsequently were used to investigate whether tumor EGFR expression level was associated with cetuximab benefit. In this analysis, a discriminatory IHC score of 200 on a scale of 0 to 300 was identified. This allowed the differentiation of a patient subgroup deriving a survival benefit from the addition of cetuximab to chemotherapy (IHC score ≥200) from one deriving little or no benefit (IHC score <200).[2]

Somatic mutations of the tyrosine kinase encoding domain of the *EGFR* gene are found in a subset of NSCLCs, consistent with a key role for aberrant EGFR function in the development of certain lung tumors.[3] In addition to presumably providing a growth or survival advantage, particular activating mutations of *EGFR* occurring in exon 18 to 21 of the gene

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also render tumor cells exquisitely sensitive to EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib or erlotinib.[4–6] The most extensively studied of such mutations and the most often occurring in NSCLC are in-frame deletions of exon 19 and the L858R missense mutation in exon 21 (classic activating mutations). Other somatic *EGFR* mutations such as exon 20 in-frame insertions[7] or the T790M missense mutation,[8,9] although they may be activating with respect to malignant transformation, do not sensitize tumors to EGFR TKI therapy, and may be associated with inherent or acquired resistance to such agents.

Early phase III studies in unselected patient populations with advanced NSCLC failed to confirm a clinical benefit associated with the addition of the EGFR TKIs gefitinib or erlotinib to standard platinum-based, first-line chemotherapy.[10–13] More recent studies have confirmed that EGFR TKI monotherapy is more effective in relation to response and progression-free survival (PFS) endpoints than chemotherapy in the first-line treatment of patients whose tumors carry activating *EGFR* mutations.[14–19]

As an EGFR antibody, the mode of action of cetuximab[20] differs from that of the EGFR TKIs. The objective of the current analysis was to assess whether the activity of cetuximab in combination with chemotherapy was modulated by the *EGFR* mutation status of tumors. In particular, clinical outcome according to treatment arm in high (IHC score ≥200) and low (IHC score <200) EGFR expression groups of FLEX study patients was investigated according to whether tumors were wild-type or mutant with respect to *EGFR*.

PATIENTS AND METHODS

FLEX Study Design and Subgroup Analysis

The FLEX study design has been reported in detail previously.[1] Briefly, patients with EGFR-expressing advanced NSCLC were assigned randomly to receive a maximum of six cycles of first-line chemotherapy comprising cisplatin and vinorelbine with or without cetuximab. After completion of chemotherapy, cetuximab monotherapy was to be administered until disease progression or the occurrence of unacceptable toxicity. The primary end point was overall survival. The clinical study was approved by an independent ethics committee at each trial center and was carried out in accordance with the Declaration of Helsinki. Written informed consent for the use of tumor tissue for molecular analysis was obtained from each patient before study entry.

Prospectively collected EGFR IHC data used initially to assess FLEX study patient eligibility were used to generate a tumor IHC score for evaluable patients (1121 of 1125 patients, 99.6%) on a scale of 0 to 300. Response then was assessed according to EGFR expression level in a subpopulation treatment effect pattern plot analysis. This led to the identification of a discriminatory IHC score threshold of 200 that could be used to distinguish a subgroup of patients who experienced a significant survival benefit from the addition of cetuximab to chemotherapy from one deriving little or no apparent benefit.[2]

EGFR Mutation Analysis

EGFR exon 18 to 21 mutation status results, determined using an EGFR29 Mutation Test Kit (DxS, Manchester, United Kingdom), were reported previously for tumors from 436 of 1125 (39%) patients from the FLEX study intention-to-treat (ITT) population.[21] This kit was designed to detect 29 previously described tumor mutations of *EGFR* including 19 different deletions of exon 19; L858R (exon 21), L861Q (exon 21), G719S/A/C (exon 18), S768I (exon 20), and T790M (exon 20) missense mutations; and three different insertions in exon 20. In the current extended analysis, the number of evaluable FLEX study patients was increased through the extraction of tumor DNA from slide-mounted tissue previously used to assess EGFR expression. *EGFR* mutation analysis on the additional samples was performed using the revised therascreen EGFR PCR Kit (QIAGEN Manchester Ltd, Manchester, United Kingdom). This system detected the same *EGFR* mutations as the previously used kit with the exception of T790M, a gatekeeper mutation associated with acquired resistance of tumors to EGFR TKIs and constitutional predisposition to NSCLC.[9,22] The assay for this mutation had been removed from the previously available kit by the manufacturer, because of technical reasons.

Statistical Analysis

The definitions of efficacy endpoints used in the present analysis including overall survival time, PFS time, objective response, and time to treatment failure (TTF) were identical to those described for the FLEX clinical study.[1] Subgroup analyses were performed using the statistical methods described previously.[2] Hazard and odds ratios (unstratified) were calculated for chemotherapy plus cetuximab versus chemotherapy alone. Evaluable patients were those for whom both a tumor EGFR IHC score and *EGFR* mutation data were available. Outcome according to treatment arm was assessed across key efficacy endpoints in ITT population subgroups defined by *EGFR* mutation status according to whether tumors expressed low (IHC score <200) or high (IHC score ≥200) levels of EGFR.

Patients for whom no tumor mutation was detected with the screening approaches used were classified for the purpose of this analysis as *EGFR* wild-type. As data on T790M mutation status were not available for the 535 samples analyzed with the therascreen EGFR PCR Kit, patients whose tumors were known from the earlier analysis to carry this EGFR TKI resistance-associated mutation (n = 4 of 436, 0.9%, as the sole identified mutation)[21] were included in the *EGFR* wild-type subgroup.

Two *EGFR* mutant subgroups were defined. The first included patients whose tumors carried any detected *EGFR* exon 18 to 20 mutation of the 28 analyzed across the full population, with the corresponding wild-type subgroup including patients with tumors in which no mutation was detected or those with T790M mutations. The second *EGFR* mutant subgroup included only patients whose tumors carried a classic *EGFR* activating mutation (exon 19 deletion or L858R missense mutation), with the corresponding comparator group being patients with tumors in which no mutation was detected or those carrying any mutation other than exon 19 deletion or L858R (exon 21). We assessed the difference in the *EGFR* mutation rate between the high and low EGFR expression groups using a two-sided Fisher's exact test. The FLEX study is registered with Clinical Trials.gov, number NCT00148798.

RESULTS

EGFR Mutation Analysis

Tumors from 971 of 1125 patients (86%) of the FLEX study ITT population were evaluable for *EGFR* mutation status, with 970 of these patients also evaluable by IHC for EGFR expression; 682 assigned to the low EGFR expression group; and 288 to the high EGFR expression group. The FLEX study profile is summarized in Supplementary Figure 1 (Supplemental digital content 1, http://links.lww.com/JTO/A538). Mutations in *EGFR* exons 18 to 21 (excluding T790M) were detected in 133 of 970 tumors (14%). The most often identified were classic activating mutations: deletions of exon 19 and the L858R missense mutation in exon 21 (124 of 133 patients, 93%; Table 1). The incidence of *EGFR* mutations was higher in tumors in the high EGFR expression group of both treatment arms (combined across arms: high: 50 of 288 [17%] versus low: 83 of 682 [12%] patients; $p = 0.041$). This difference was reflected in a higher median IHC score for tumors with, versus those without, mutations (Fig. 1).

TABLE 1. Frequency of Particular *EGFR* Mutations According to EGFR Expression Group

EGFR mutation, *n*	Low EGFR Expression[a] (*n* = 682)	High EGFR Expression[b] (*n* = 288)	Total (*n* = 970)
Deletion exon 19	53	27	80
L858R	24	20	44
G719X	2	2	4
L861Q	2	1	3
Insertion exon 20	2	0	2
Total, *n* (%)	83 (12)	50 (17)	133 (14)

[a]Immunohistochemistry score < 200.
[b]Immunohistochemistry score ≥ 200.

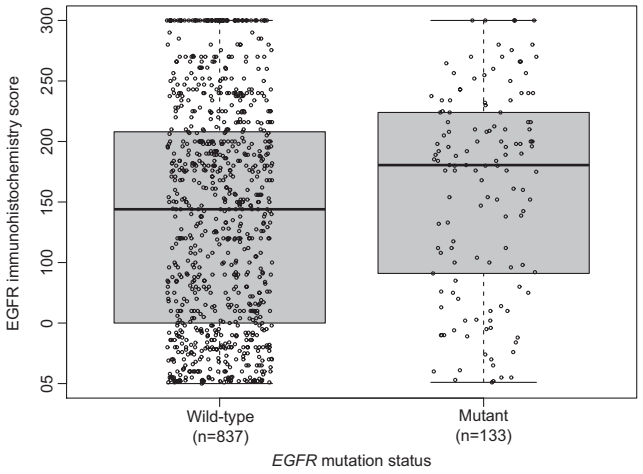

FIGURE 1. Distribution of EGFR immunohistochemistry scores according to *EGFR* mutation status. Upper/lower boundaries of each box plot represent the 25th/75th percentile; horizontal lines within the box denote median values.

Treatment Efficacy

Treatment outcomes, as indicated by hazard and odds ratios for efficacy endpoints according to *EGFR* mutation status and EGFR expression group, are summarized in Table 2. Relative treatment outcomes across efficacy endpoints in the *EGFR* mutation evaluable population ($n = 971$) were similar to those reported for the overall ITT population ($n = 1125$), although the estimated treatment effect of adding cetuximab to chemotherapy for overall survival was somewhat less favorable in the *EGFR* mutation evaluable compared with the ITT population for all patients and for those in both the low and high EGFR expression subgroups.

Median survival was markedly longer in patients whose tumors carried *EGFR* mutations (any detected) compared with those with *EGFR* wild-type tumors in both the chemotherapy plus cetuximab (17.3 versus 9.6 months) and chemotherapy alone treatment groups (19.8 versus 9.6 months). This was also the case when considering patients whose tumors carried classic activating mutations (deletion exon 19 or L858R) versus all other patients (Table 3). Median PFS and TTF also were longer in patients with *EGFR* mutant compared with wild-type tumors (Supplementary Tables 1 and 2; Supplemental digital content 2, http://links.lww.com/JTO/A539). In addition, the response rate was markedly higher in patients whose tumors carried *EGFR* mutations (all and classic activating; Supplementary Table 3, Supplemental digital content 2, http://links.lww.com/JTO/A539) compared with patients whose tumors were *EGFR* wild-type (or wild-type plus nonclassic mutation) in both the chemotherapy plus cetuximab (any mutation, 54.5% versus wild-type, 32.9%) and chemotherapy alone treatment groups (any mutation, 35.8% versus wild-type 27.0%).

For patients with *EGFR* wild-type tumors, a survival benefit associated with the addition of cetuximab to chemotherapy was apparent in the high EGFR expression group, (hazard ratio [HR] 0.76, 95% confidence interval [CI] 0.57–1.00; Fig. 2). When patients with nonclassic mutations were included in this wild-type group, a similar cetuximab benefit was seen (HR 0.79, 95% CI 0.60–1.04). No benefit was apparent in the low EGFR expression group for patients with *EGFR* wild-type tumors (HR 0.98, 95% CI 0.82–1.18). PFS and TTF were similar across treatment and EGFR expression groups for patients with *EGFR* wild-type tumors. However, in the *EGFR* wild-type, high EGFR expression group, the response rate was higher for patients in the chemotherapy plus cetuximab compared with chemotherapy alone treatment group (39.0% versus 27.0%, respectively, odds ratio 1.73, 95% CI 1.00–3.00). Again this effect was similar when patients whose tumors carried nonclassic mutations were included in the wild-type group (39.5% versus 27.4%, respectively, odds ratio 1.74, 95% CI 1.01–2.99). Response rates in the *EGFR* wild-type and wild-type plus nonclassic mutation, low EGFR expression groups were similar across the treatment arms.

In the high EGFR expression group, patients with tumor *EGFR* mutations may also have derived a survival benefit from the addition of cetuximab to chemotherapy, including when this group was restricted to those with classic activating mutations (Tables 2 and 3, Fig. 3). Conversely, in the low

TABLE 2. Outcome According to Tumor EGFR Expression Level and *EGFR* Mutation Status

	Overall survival	PFS	TTF	Response
Population[a]	HR[b] (95% CI)	HR[b] (95% CI)	HR[b] (95% CI)	Odds ratio[b] (95% CI)
ITT				
All patients, *n* = 1125	0.87 (0.76–1.00)	0.94 (0.82–1.08)	0.86 (0.76–0.97)	1.39 (1.08–1.78)
Low EGFR IHC score, *n* = 776	0.99 (0.84–1.16)	0.98 (0.83–1.15)	0.9 (0.78–1.04)	1.15 (0.85–1.56)
High EGFR IHC score, *n* = 345	0.73 (0.58–0.93)	0.86 (0.68–1.09)	0.78 (0.63–0.97)	2.04 (1.30–3.19)
EGFR mut evaluable				
All patients, *n* = 971	0.95 (0.82–1.09)	0.97 (0.84–1.12)	0.86 (0.75–0.98)	1.42 (1.09–1.87)
Low EGFR IHC score, *n* = 682	1.04 (0.87–1.23)	0.99 (0.83–1.17)	0.89 (0.76–1.04)	1.23 (0.89–1.71)
High EGFR IHC score, *n* = 288	0.78 (0.60–1.01)	0.91 (0.70–1.18)	0.8 (0.63–1.02)	1.88 (1.16–3.05)
EGFR wt[c]				
All patients, *n* = 838	0.91 (0.78–1.06)	1.02 (0.87–1.19)	0.9 (0.79–1.04)	1.33 (0.99–1.78)
Low EGFR IHC score, *n* = 599	0.98 (0.82–1.18)	1.03 (0.86–1.24)	0.92 (0.78–1.09)	1.16 (0.82–1.66)
High EGFR IHC score, *n* = 238	0.76 (0.57–1.00)	0.96 (0.72–1.29)	0.86 (0.66–1.12)	1.73 (1.00–3.00)
EGFR mut (any[c])				
All patients, *n* = 133	1.22 (0.79–1.89)	0.7 (0.48–1.04)	0.64 (0.44–0.91)	2.15 (1.07–4.31)
Low EGFR IHC score, *n* = 83	1.53 (0.89–2.63)	0.71 (0.43–1.17)	0.64 (0.41–1.02)	1.75 (0.71–4.29)
High EGFR IHC score, *n* = 50	0.86 (0.40–1.84)	0.69 (0.37–1.30)	0.59 (0.33–1.08)	3.27 (1.01–10.6)
EGFR no del exon 19/L858R				
All patients, *n* = 847	0.92 (0.79–1.06)	1.01 (0.87–1.18)	0.9 (0.78–1.03)	1.36 (1.01–1.83)
Low EGFR IHC score, *n* = 605	0.97 (0.81–1.17)	1.01 (0.85–1.22)	0.91 (0.77–1.07)	1.2 (0.85–1.71)
High EGFR IHC score, *n* = 241	0.79 (0.60–1.04)	0.98 (0.73–1.31)	0.86 (0.66–1.12)	1.74 (1.01–2.99)
EGFR mut, del exon 19 or L858R				
All patients, *n* = 124	1.28 (0.81–2.03)	0.73 (0.48–1.09)	0.67 (0.46–0.97)	1.82 (0.89–3.73)
Low EGFR IHC score, *n* = 77	1.82 (1.01–3.26)	0.79 (0.47–1.32)	0.71 (0.44–1.15)	1.36 (0.54–3.43)
High EGFR IHC score, *n* = 47	0.74 (0.34–1.60)	0.65 (0.34–1.23)	0.59 (0.32–1.09)	3.16 (0.95–10.5)

[a]Low EGFR IHC score <200; high EGFR IHC score ≥200.
[b]Hazard and odds ratios are for chemotherapy + cetuximab versus chemotherapy (ITT population analysis, stratified; subgroup analyses, unstratified).
[c]T790M not considered/assessed.
HR, hazard ratio; PFS, progression-free survival; TTF, time to treatment failure; ITT, intention-to-treat; EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; mut, mutation; wt, wild-type; del, deletion; CI, confirdence interval.

EGFR expression group of both mutation subgroups, the addition of cetuximab to chemotherapy seemed to be associated with shorter survival compared with chemotherapy alone (any detected: median 12.7 versus 19.8 months, HR 1.53, 95% CI 0.89–2.63; Fig. 3; classic activating: median 12.3 versus 23.8 months, respectively, HR 1.82, 95% CI 1.01–3.26).

Somewhat paradoxically, for both mutation subgroups, the addition of cetuximab to chemotherapy was associated with longer PFS and TTF in both low and high EGFR expression groups. Similarly, response rates for patients with EGFR mutations were higher in the chemotherapy plus cetuximab compared with chemotherapy treatment groups in both the low and high EGFR expression groups, although this difference was most apparent in the high EGFR expression group (Table 2, Supplementary Tables 1–3, Supplemental digital content 2, http://links.lww.com/JTO/A539).

DISCUSSION

Tumors arise through a stepwise process of somatic mutation and clonal selection. During this process, advantageous driver lesions, such as point mutations, translocations or gene amplifications may confer a growth or survival advantage to the developing tumor cells.[23] In relation to the tailoring of therapy, the presence of a particular driver or activating mutation may be associated with the sensitivity of a tumor to anticancer agents targeting the protein product of the mutated gene. This paradigm has been demonstrated clearly in the case of EGFR[4,6] and ALK[24] TKI therapy in NSCLC. For patients with advanced disease whose tumors carry activating *EGFR* mutations, standard platinum-based doublet chemotherapy does not seem to be the optimum first-line treatment strategy, with randomized trials showing higher overall response rates and longer PFS associated with EGFR TKI monotherapy in this patient group.[14–16,18] In contrast, for patients whose tumors do not carry such mutations, there is little evidence that EGFR TKI therapy confers a substantial benefit in the first-line setting.[25]

The FLEX study demonstrated a survival advantage associated with the addition of cetuximab to cisplatin and vinorelbine first-line chemotherapy in patients with EGFR-expressing advanced NSCLC. In an attempt to identify predictive biomarkers which could be used to tailor cetuximab therapy to patient subgroups most likely to benefit, several candidate biomarkers were investigated in retrospective

TABLE 3. Median Overall Survival According to Treatment Arm, *EGFR* Mutation Group, and EGFR Expression Group

	Chemotherapy + cetuximab		Chemotherapy alone	
Population[a]	***n***	**Median, months (95% CI)**	***n***	**Median, months (95% CI)**
ITT				
All patients	557	11.3 (9.4–12.4)	568	10.1 (9.1–10.9)
Low EGFR IHC score	377	9.8 (8.9–12.2)	399	10.3 (9.2–11.5)
High EGFR IHC score	178	12.0 (10.2–15.2)	167	9.6 (7.6–10.6)
EGFR mut evaluable				
All patients	482	11.2 (9.2–12.3)	489	10.2 (9.2–11.1)
Low EGFR IHC score	333	9.7 (8.8–12.2)	349	10.3 (9.2–12.1)
High EGFR IHC score	148	11.5 (10.0–15.0)	140	9.6 (7.7–10.8)
EGFR wt[b]				
All patients	416	9.6 (8.8–11.4)	422	9.6 (8.7–10.3)
Low EGFR IHC score	292	9.4 (8.2–11.9)	307	10.0 (9.0–11.3)
High EGFR IHC score	123	10.2 (8.0–12.0)	115	8.5 (7.3–10.2)
EGFR mut (any[b])				
All patients	66	17.3 (12.7–21.0)	67	19.8 (15.2–NR)
Low EGFR IHC score	41	12.7 (8.7–20.1)	42	19.8 (10.3–NR)
High EGFR IHC score	25	21.9 (16.4–NR)	25	19.5 (11.8–NR)
EGFR no del exon 19/L858R				
All patients, *n* = 847	418	9.7 (8.8–11.4)	429	9.6 (8.7–10.3)
Low EGFR IHC score	293	9.4 (8.2–12.0)	312	10.0 (8.9–11.3)
High EGFR IHC score	124	10.2 (8.0–12.0)	117	8.7 (7.4–10.3)
EGFR mut, del exon 19 or L858R				
All patients	64	17.5 (12.7–21.9)	60	20.7 (15.8–NR)
Low EGFR IHC score	40	12.3 (8.7–20.1)	37	23.8 (12.1–NR)
High EGFR IHC score	24	21.9 (17.5–NR)	23	19.5 (11.8–NR)

[a]Low EGFR IHC score < 200; high EGFR IHC score ≥ 200.
[b]T790M not considered/assessed.
ITT, intention-to-treat; EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; mut, mutation; wt, wild-type; NR, not reached; del, deletion; CI, confirdence interval.

analyses.[21] Comparisons of treatment outcome according to *KRAS* mutation status, *EGFR* mutation status, *EGFR* copy number, or PTEN expression status provided no indication that these biomarkers might be of predictive value in this setting. However, in a further analysis based on prospectively collected FLEX study IHC data, high EGFR expression was shown to be a tumor biomarker that could predict a survival benefit associated with the addition of cetuximab to chemotherapy.[2] The current analysis was designed to investigate whether the survival benefit demonstrated in the FLEX study for the addition of cetuximab to chemotherapy in patients whose tumors expressed high levels of EGFR (IHC score ≥200) was affected by *EGFR* mutation status.

To carry out this assessment, the previous analysis of *EGFR* mutation status in 39% of patients from the FLEX study was extended to include 86% of the ITT population.[21] The mutation rate in this extended group, based on all detected mutations but excluding T790M, was 14%, with 93% of identified mutations being classic activating mutations as represented by exon 19 deletions and the L858R missense mutation. Other mutations identified included exon 20 insertions (2%), which have been associated with a lack of sensitivity to EGFR TKIs, and G179X (3%) and L861Q mutations (2%), which seem to be associated with a degree of sensitivity to EGFR TKIs.[7,26] In relation to lung cancer etiology, the presence of all such mutations at baseline in NSCLC tissues, regardless of whether they confer EGFR TKI sensitivity or not, is consistent with their role as gain-of-function driver mutations. For the purposes of this analysis, patients with these mutations were therefore considered in the first instance as one overall *EGFR* mutant group. However, as the classic *EGFR* activating mutations seem to be those associated most strongly with TKI sensitivity, outcome in patients whose tumors had this type of mutation was also separately compared with that of patients not having such mutations (*EGFR* wild-type plus nonclassic mutations group).

A tendency for tumors with *EGFR* mutations (apart from T790M) to express higher levels of EGFR compared with wild-type tumors was apparent. Similar associations have been reported previously in some[27,28] but not other[29,30] NSCLCs studies. Also consistent with previous studies in which patients with advanced NSCLC received chemotherapy, survival in both treatment groups of the FLEX study was longer for patients whose tumors carried *EGFR* exon 18 to 21 mutations.[31–33] This was the case for both any detected and classic activating *EGFR* mutation groups compared with their corresponding nonmutant groups. Whether this reflects a direct biological effect of *EGFR* mutation in NSCLC cells or

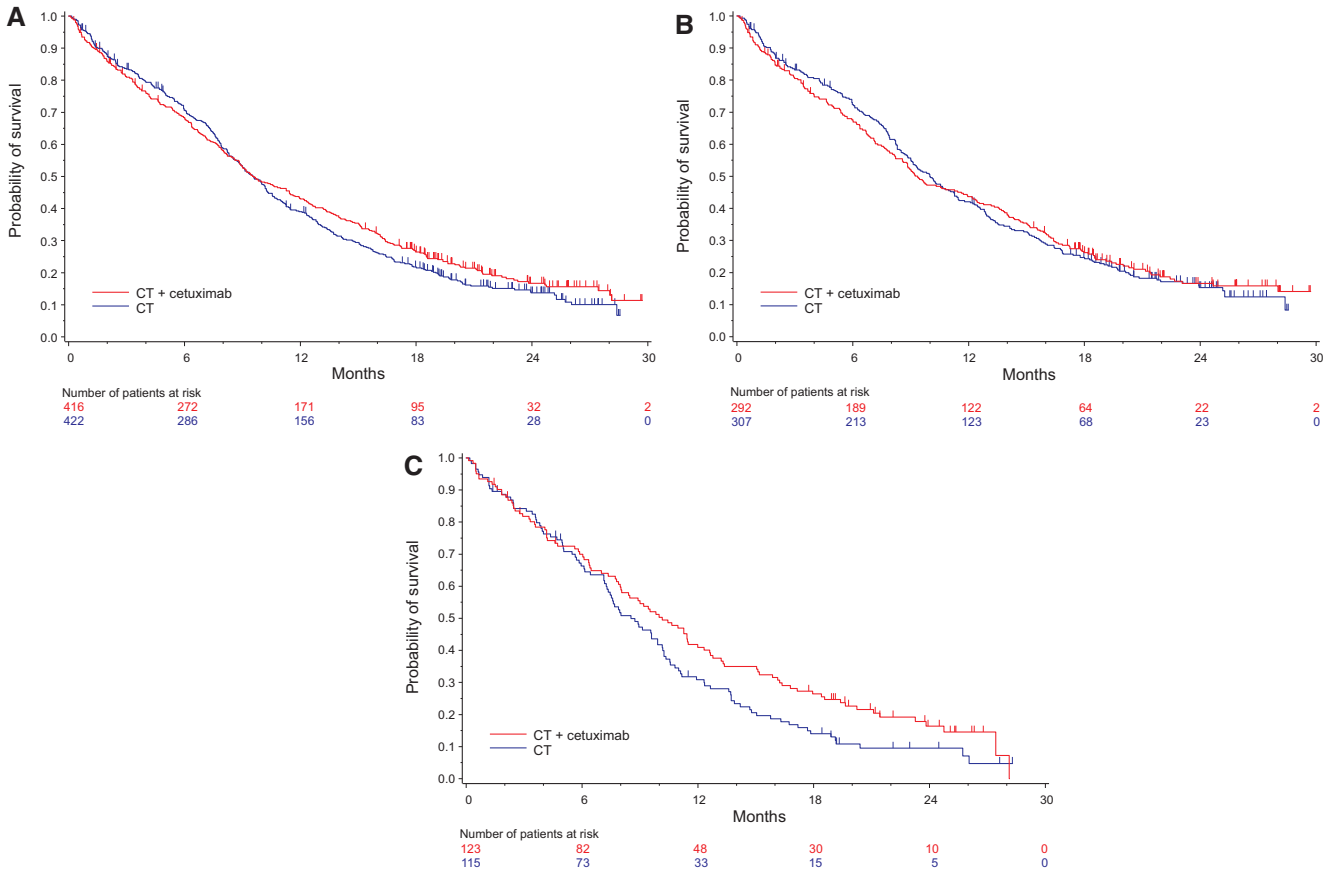

FIGURE 2. Kaplan-Meier plots for overall survival: *EGFR* wild-type subgroups. Survival according to treatment group in evaluable FLEX study patients with *A, EGFR* wild-type tumors (including T790M) and (*B*) low (immunohistochemistry score <200) and (C) high (immunohistochemistry score ≥200) EGFR expression subgroups of this population.

an association of *EGFR* mutation with other factors linked to good prognosis is not clear.

The previously reported analysis of treatment outcome according to tumor EGFR expression level in FLEX study patients suggested that the survival benefit associated with the addition of cetuximab to chemotherapy was limited to patients with high EGFR expression, as defined by an IHC score of ≥200. In the current analysis, this survival benefit was clearly apparent for patients in the high EGFR expression group of the *EGFR* wild-type population. The magnitude of benefit, as reflected by the HR (0.76, 95% CI 0.57–1.00), was similar to that reported for the high expression group of the ITT population (0.73, 95% CI 0.58–0.93). In contrast, no such benefit was apparent in the low EGFR expression group of the *EGFR* wild-type population. A similar cetuximab survival benefit also was evident when the *EGFR* wild-type population was extended to include patients with nonclassic activating mutations (HR 0.79, 95% CI 0.60–1.04). The demonstration of treatment benefit in patients with *EGFR* wild-type tumors therefore differentiates cetuximab activity in this setting from that of the EGFR TKIs.

The relatively small number of patients whose tumors carried *EGFR* mutations precluded the drawing of definitive conclusions regarding the effect of adding cetuximab to chemotherapy in these groups. However, although such patients in the high EGFR expression group may have derived some measure of survival benefit from cetuximab, patients with tumor *EGFR* mutations in the low EGFR expression group who received chemotherapy plus cetuximab had shorter overall survival than those receiving chemotherapy alone. Although it cannot be excluded that these survival differences were related to imbalances between treatment groups in the administration of post-study EGFR TKI therapy, this effect was particularly apparent when considering those with classic activating mutations (low EGFR expression group: median survival 12.3 versus 23.8 months, respectively, HR 1.82, 95% CI 1.01–3.26; high EGFR expression group median survival 21.9 versus 19.5 months, HR 0.74, 95% CI 0.34–1.60).

Patients in the high EGFR expression group who received chemotherapy plus cetuximab seemed to have a higher response rate regardless of *EGFR* mutation status compared with those receiving chemotherapy alone, with the biggest differences between treatment arms seen in the *EGFR* mutant subgroups. Somewhat in contrast to the overall survival data, cetuximab-associated improvements in PFS and TTF were mainly seen in the *EGFR* mutant subgroups, with similar degrees of benefit suggested for the low and high EGFR expression groups. These data suggesting cetuximab

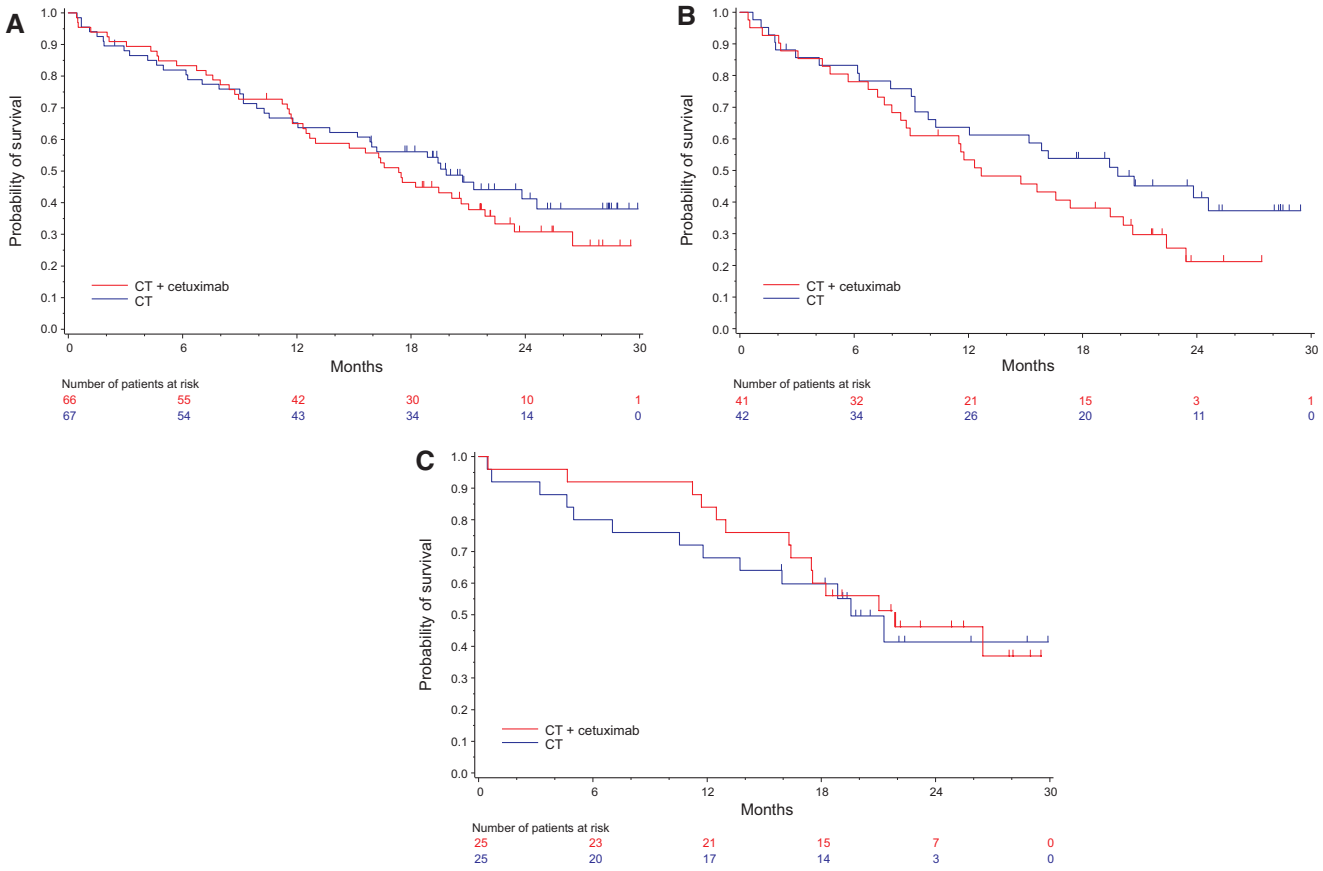

FIGURE 3. Kaplan-Meier plots for overall survival: *EGFR* mutant subgroups. Survival according to treatment group in evaluable FLEX study patients with (*A*) *EGFR* mutant tumors (all detected mutations) and (*B*) low (immunohistochemistry score <200) and (C) high (immunohistochemistry score ≥200) EGFR expression subgroups of this population.

activity in tumors with *EGFR* mutations are consistent with findings from experimental models showing that cetuximab can induce enhanced degradation of mutant *EGFRs* compared with wild-type receptors.[34]

Cetuximab is not currently approved for the treatment of advanced NSCLC. To gain such approval, further supportive clinical trial data would be required. This may be provided by the large, ongoing phase III SWOG S0819 trial (NCT00946712), exploring carboplatin, paclitaxel, and if appropriate, bevacizumab, with versus without cetuximab as first-line treatment for stage IV or recurrent NSCLC.[35] A secondary objective of this trial is the prospective validation of *EGFR* fluorescence in situ hybridization status as a predictive biomarker for cetuximab activity.

In summary, the current analysis showed that FLEX study patients with advanced NSCLC expressing high levels of EGFR who did not have activating mutations of *EGFR* in their tumors derived a survival benefit from the addition of cetuximab to first-line chemotherapy. Patients in the high EGFR expression group whose tumors carried *EGFR* mutations (all detected or classic activating only) may have derived a similar benefit, although definitive conclusions cannot be drawn because of small patient numbers. High EGFR expression remains a potentially useful tumor biomarker in relation to predicting a survival benefit associated with the addition of cetuximab to first-line chemotherapy for NSCLC.

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REFERENCES

1. Pirker R, Pereira JR, Szczesna A, et al.; FLEX Study Team. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. *Lancet* 2009;373:1525–1531.
2. Pirker R, Pereira JR, von Pawel J, et al. EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the phase 3 FLEX study. *Lancet Oncol* 2012;13:33–42.
3. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007;7:169–181.

4. Eck MJ, Yun CH. Structural and mechanistic underpinnings of the differential drug sensitivity of EGFR mutations in non-small cell lung cancer. *Biochim Biophys Acta* 2010;1804:559–566.
5. Gazdar AF. Activating and resistance mutations of EGFR in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. *Oncogene* 2009;28(Suppl 1):S24–S31.
6. Pao W, Chmielecki J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. *Nat Rev Cancer* 2010;10:760–774.
7. Yasuda H, Kobayashi S, Costa DB. EGFR exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications. *Lancet Oncol* 2012;13:e23–e31.
8. Su KY, Chen HY, Li KC, et al. Pretreatment epidermal growth factor receptor (EGFR) T790M mutation predicts shorter EGFR tyrosine kinase inhibitor response duration in patients with non-small-cell lung cancer. *J Clin Oncol* 2012;30:433–440.
9. Suda K, Onozato R, Yatabe Y, Mitsudomi T. EGFR T790M mutation: a double role in lung cancer cell survival? *J Thorac Oncol* 2009;4:1–4.
10. Gatzemeier U, Pluzanska A, Szczesna A, et al. Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non-small-cell lung cancer: the Tarceva Lung Cancer Investigation Trial. *J Clin Oncol* 2007;25:1545–1552.
11. Giaccone G, Herbst RS, Manegold C, et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial–INTACT 1. *J Clin Oncol* 2004;22:777–784.
12. Herbst RS, Giaccone G, Schiller JH, et al. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial–INTACT 2. *J Clin Oncol* 2004;22:785–794.
13. Herbst RS, Prager D, Hermann R, et al.; TRIBUTE Investigator Group. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. *J Clin Oncol* 2005;23:5892–5899.
14. Mitsudomi T, Morita S, Yatabe Y, et al.; West Japan Oncology Group. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* 2010;11:121–128.
15. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009;361:947–957.
16. Rosell R, Carcereny E, Gervais R, et al.; Spanish Lung Cancer Group in collaboration with Groupe Français de Pneumo-Cancérologie and Associazione Italiana Oncologia Toracica. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012;13:239–246.
17. Zhou C, Wu YL, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* 2011;12:735–742.
18. Bria E, Milella M, Cuppone F, et al. Outcome of advanced NSCLC patients harboring sensitizing EGFR mutations randomized to EGFR tyrosine kinase inhibitors or chemotherapy as first-line treatment: a meta-analysis. *Ann Oncol* 2011;22:2277–2285.
19. Sequist LV, Yang JC, Yamamoto N, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* 2013;31:3327–3334.
20. Li S, Schmitz KR, Jeffrey PD, Wiltzius JJ, Kussie P, Ferguson KM. Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. *Cancer Cell* 2005;7:301–311.
21. O'Byrne KJ, Gatzemeier U, Bondarenko I, et al. Molecular biomarkers in non-small-cell lung cancer: a retrospective analysis of data from the phase 3 FLEX study. *Lancet Oncol* 2011;12:795–805.
22. Oxnard GR, Miller VA, Robson ME, et al. Screening for germline EGFR T790M mutations through lung cancer genotyping. *J Thorac Oncol* 2012;7:1049–1052.
23. Greaves M, Maley CC. Clonal evolution in cancer. *Nature* 2012;481:306–313.
24. Gandhi L, Jänne PA. Crizotinib for ALK-rearranged non-small cell lung cancer: a new targeted therapy for a new target. *Clin Cancer Res* 2012;18:3737–3742.
25. Lorusso V, Silvestris N, Marech I. TORCH study: how much longer should we continue to use erlotinib in unselected patients with non-small-cell lung cancer? *J Clin Oncol* 2013;31:288–289.
26. Wu JY, Yu CJ, Chang YC, Yang CH, Shih JY, Yang PC. Effectiveness of tyrosine kinase inhibitors on "uncommon" epidermal growth factor receptor mutations of unknown clinical significance in non-small cell lung cancer. *Clin Cancer Res* 2011;17:3812–3821.
27. Liang Z, Zhang J, Zeng X, Gao J, Wu S, Liu T. Relationship between EGFR expression, copy number and mutation in lung adenocarcinomas. *BMC Cancer* 2010;10:376.
28. Pinter F, Papay J, Almasi A, et al. Epidermal growth factor receptor (EGFR) high gene copy number and activating mutations in lung adenocarcinomas are not consistently accompanied by positivity for EGFR protein by standard IHC. *J Mol Diagn* 2008;10:160–168.
29. Cappuzzo F, Hirsch FR, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2005;97:643–655.
30. Tsao AS, Tang XM, Sabloff B, et al. Clinicopathologic characteristics of the EGFR gene mutation in non-small cell lung cancer. *J Thorac Oncol* 2006;1:231–239.
31. Bell DW, Lynch TJ, Haserlat SM, et al. Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. *J Clin Oncol* 2005;23:8081–8092.
32. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* 2005;23:5900–5909.
33. Kalikaki A, Koutsopoulos A, Hatzidaki D, et al. Clinical outcome of patients with non-small cell lung cancer receiving front-line chemotherapy according to EGFR and K-RAS mutation status. *Lung Cancer* 2010;69:110–115.
34. Doody JF, Wang Y, Patel SN, et al. Inhibitory activity of cetuximab on epidermal growth factor receptor mutations in non small cell lung cancers. *Mol Cancer Ther* 2007;6:2642–2651.
35. Redman MW, Crowley JJ, Herbst RS, Hirsch FR, Gandara DR. Design of a phase III clinical trial with prospective biomarker validation: SWOG S0819. *Clin Cancer Res* 2012;18:4004–4012.